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OPIOID STRUCTURE: LIPID THERMOTROPIC BEHAVIOUR CORRELATION STUDY ON A SERIES OF DPPC LIPOSOMES CONTAINING OPIOIDS

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ABSTRACT

The effect of morphine, naloxone, codeine and ethylmorphine on the thermotropic properties of dipalmitoylphosphatidylcholine (DPPC) liposomes has been examined by differential scanning calorimetry. All the drugs in this series eliminated the DPPC pre-transition peak. Moreover, both codeine and ethylmorphine showed a noticeable shift of the gel–liquid crystal transition peak T_m towards lower values, along with a broadening of the melting peak and a slight variation in the enthalpy of melting. The different thermotropic behaviour among this series of opioids could be explained by structural features such as the different substitution on the alcohol function of the aromatic ring of the benzomorphane moiety.

INTRODUCTION

In addition to functioning as physical and chemical barriers separating aqueous compartments, biological membranes are involved in many regulatory processes. Several roles for the lipid constituents of such membranes in mediating these processes have been proposed and/or demonstrated. Among these, a feature of great interest in the elucidation of the molecular mechanism of drug–receptor interaction is the binding affinity of some drugs to their receptors.

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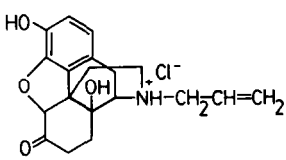
Although the successful isolation, purification and preliminary characterization of opioid receptors has been reported, their chemical composition is still uncertain [1–3]. Like other membrane receptors, the opioid receptor has a protein nature; however, there is growing evidence of the involvement of phospho- and glycolipids in its structure and function [4–6].

Studies on the structure-activity relationship of opioid substances have demonstrated that hydrophobicity is an important factor influencing their ability to cross the blood–brain barrier and their interaction with the lipidic components of opioid receptors [7]. One of the most likely kinds of interaction between membrane lipids and opioids probably involves membrane fluidity changes. Thus, it has been found that microviscosity affects the binding of opioids to their receptors by modifying the number of binding sites and therefore the affinity of the opioid ligands [8,9].

In our attempt to build evidence in favour of such an hypothesis [10,11], in the present work we have been using liposomes of pure dipalmitoylphosphatidylcholine (DPPC) as a biological membrane model to study the interaction of four opioid substances (see Fig. 1) with this major lipid component of biological membranes. Thus, following its common use in other aspects of membrane research [12–14], we have applied high sensitivity scanning calorimetry to examine how the presence of several opioids differently affects the peak temperature of the gel–liquid crystal phase transition and its associated enthalpy changes ocurring in DPPC liposomes. The main aim was to correlate the thermotropic behaviour of these model phospholipid membranes with structural features of a series of opioid substances.



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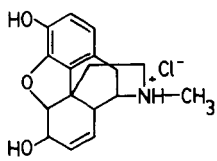
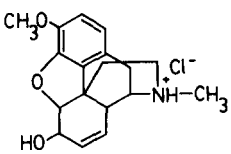
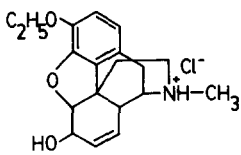


MORPHINE . HCl



CODEINE . HCl



ETHYLMORPHINE . HCl

Fig. 1. Chemical structure of naloxone, morphine, codeine and ethylmorphine.

MATERIALS AND METHODS

Chemicals

Synthetic L-α-dipalmitoylphosphatidylcholine was obtained from Fluka AG, puriss. grade product (approx. 99.6%); however, its purity was checked by two-dimensional thin layer chromatography on silica gel plates.

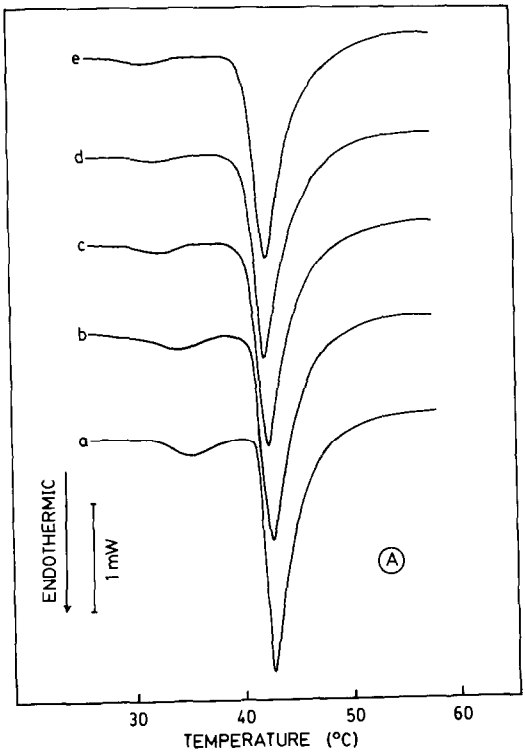
We are grateful for the receipt of the following drugs as gifts: morphine and naloxone (Laboratorios Abelló S.A., Spain), codeine (Bayer S.A., Spain) and ethylmorphine (Uquifa Laboratorios, Spain). All drugs were received as hydrochlorides and were subsequently recrystallized from methanol. Their purity was assessed by elemental analysis performed at the Laboratory of Microanalysis of the Biological Organic Chemistry Department (C.I.D.) (C.S.I.C.), Barcelona.

Preparation of liposomes

Stock solutions of both DPPC in $HCCl_3$ and drugs in MeOH were prepared and mixed in the appropriate ratio in order to obtain samples containing 8 mg of DPPC and different amounts of opioids. The solvent was removed under nitrogen flow at 35–40 °C in a rotatory evaporator. The residual traces of solvents were eliminated by lyophilization of the lipidic film for three hours. Liposomes were formed by adding 200 μl of distilled water to the film, heating the hydrated samples in a water bath at 60 °C and mixing strongly (using a vortex) three times during 1 min. In order to obtain homogeneous liposomes, samples were further stabilized by keeping them at 55 °C in a floating water bath for 1 h. The liposomes formed were immediately submitted to thermal analysis. The phosphorous content of the samples was determined according to the Barlett method [15].

Differential scanning calorimetry

The thermal transitions of the liposome preparations were measured on a Mettler TA 3000 system equipped with a TC 10 TA control processor and a DSC 30 calorimetric cell. Samples were prepared by loading 120 μl of the lipid dispersion, always containing an average of 5 mg DPPC, into aluminium pans of 160 μl capacity. The sealed samples were submitted to calorimetric measurements performed in the temperature range 10–55 °C at a scanning rate of 2 °C min^{-1} and a sensitivity of 1.7 mW of full scale. Water was always present in the reference pan. The system was temperature and heat flow calibrated using palmitic and lauric acids. The enthalpic changes were calculated from the main DSC peak areas using a planimeter and an integration computer program. For each sample at least four heating and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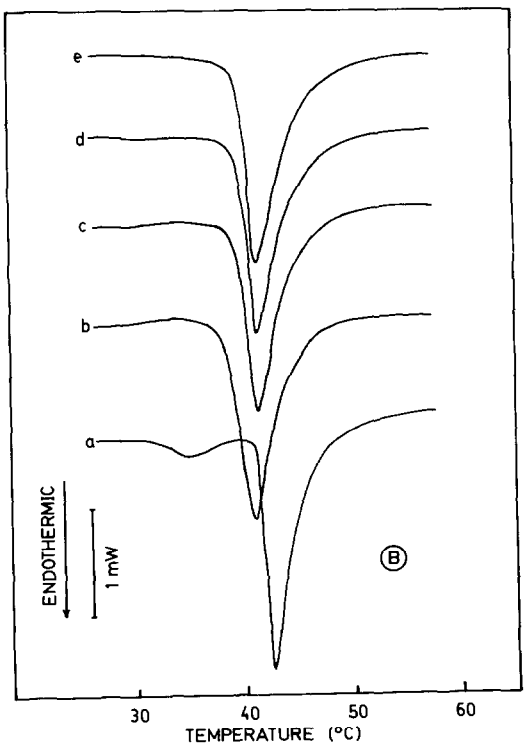
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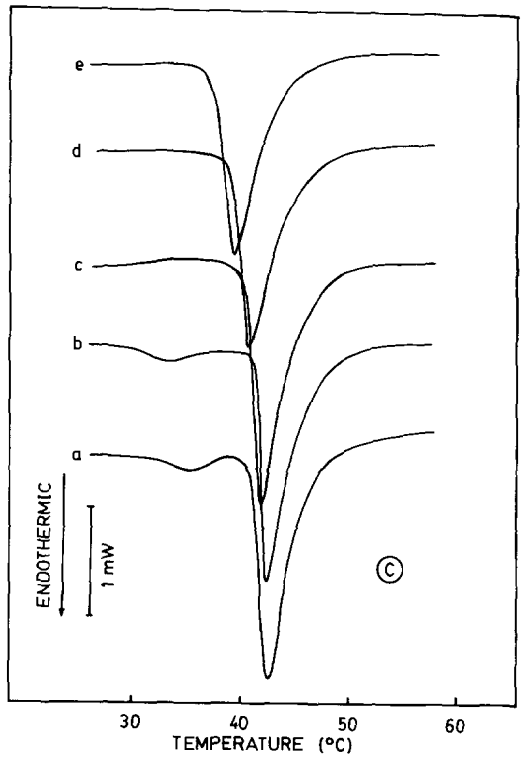

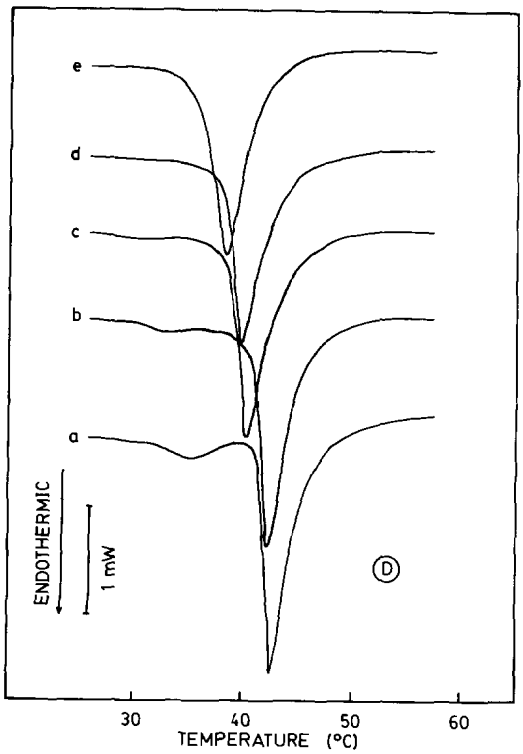

Fig. 2. Typical DSC heating profiles of 1,2-dipalmitoyllecithin liposomes containing (A) morphine hydrochloride, (B) naloxone hydrochloride, (C) codeine hydrochloride and (D) ethylmorphine hydrochloride in the following mole fractions: a, 0; b, 0.09; c, 0.24; d, 0.50; e, 0.70.

cooling cycles were recorded to ensure the reproducibility of the thermotropic behaviour.

RESULTS AND DISCUSSION

The DSC profiles for pure DPPC dispersed in water and in the presence of different amounts of a series of opioid drugs are shown in Fig. 2A–D.

For the DPPC–morphine hydrochloride system, there were no noticeable differences in the gel–liquid crystal transition peaks which have practically constant temperatures. However, the DPPC pre-transition peak gradually reduced until it completely disappeared at high drug mole-fraction values. Similar results have been observed for the DPPC–naloxone hydrochloride system. Thus, no clear drug concentration–thermotropic behaviour relationship could be established apart from a slight reduction in both T_m and ΔH; in addition, the pre-transition peak disappeared when drug concentration was increased in the liposome preparations.

When the drug concentration was increased in the DPPC–codeine hydrochloride system, the pre-transition peak disappeared at very low molecular fractions ($x > 0.09$). Furthermore, a remarkable shift towards lower temperatures was also observed for the main transition peak. In addition, by increasing the drug concentration, the associated ΔH values remained practically constant. In the DPPC–ethylmorphine hydrochloride system, similar results were obtained to those reported by us for the DPPC–codeine system [10]. Thus, low molar fractions of drug ($x = 0.09$) were able to suppress the DPPC pre-transition peak and a shift towards lower temperatures could be detected. When the drug concentration was increased, this gradual T_m reduction was accompanied by a small broadening of the main DSC peak along with no meaningful ΔH changes.

Despite the pronounced structural similarities among the opioids, shown in Fig. 1, we wanted to demonstrate that the different effects which such drugs exert on the thermotropic behaviour of the DPPC liposomes can be attributed to some particular structural features. It is a known fact that even small differences in the substituent units lead to different interaction patterns between certain drugs and phospholipid vesicles. For opioid drugs, this has been studied by Cater et al. [16]. By examining the effect of structurally related morphine compounds upon the lipid endothermic phase transition, they concluded that by changing the length of the alkyl chain of the alcohol residue present on the C-7 of the morphine core, differences in the transition temperature of lecithin–water systems can be observed.

Among the series of opioid drugs examined, codeine shows the most noticeable effect on the structure of the DPPC bilayer. The results obtained suggest that codeine fluidifies the DPPC bilayer by immersing its molecules

into an ordered structure, behaving in a manner similar to that which we have previously observed with ethylmorphine.

In addition, all these drugs were found to affect the T_m to different extents; however, no significant lowering of the heat of the gel–liquid crystal phase transition could be observed. This fact is an indication that these effects are due to a superficial electrostatic interaction with no deep interference in the packing of the lipid chains [17,18]. In summary, these results present reasonable evidence that a major disruption of the lipid-chain-packing by interdigitation of the drugs does not occur, although superficial penetration within the area of the polar groups of the lipid may still be possible.

Because of the very similar molecular features of morphine and naloxone (Fig. 1), similar physico-chemical properties can be expected. Accordingly,

TABLE 1

Main transition peak temperatures T_m (°C) of DPPC liposomes containing different molar fractions of opioids. The mean values of at least four DSC heating cycles are given

Mole fraction	T_m			
	Morphine	Naloxone	Codeine	Ethylmorphine
0.00	42.1	42.1	42.1	42.1
0.03	41.3	41.2	41.9	41.9
0.06	41.5	40.5	41.9	41.8
0.09	41.6	40.1	41.7	41.5
0.12	42.1	40.5	41.5	41.1
0.24	41.1	41.0	41.2	40.3
0.33	41.6	41.1	40.9	38.4
0.50	41.6	40.7	40.4	38.1
0.75	42.0	40.9	38.9	37.3

TABLE 2

Main transition enthalpy changes ΔH (kcal, mol^{-1}) of DPPC liposomes containing different molar fractions of opioids. The mean values of at least four DSC cycles are given

Mole fraction	ΔH			
	Morphine	Naloxone	Codeine	Ethylmorphine
0.00	8.6	8.6	8.6	8.6
0.03	7.2	7.9	8.3	8.0
0.06	7.3	7.4	8.0	7.9
0.09	7.4	7.2	8.1	7.7
0.12	7.9	7.2	8.3	7.5
0.24	6.8	7.1	8.5	8.1
0.33	7.3	7.1	8.3	8.2
0.50	7.8	7.2	7.9	7.3
0.75	7.1	7.5	7.8	8.4

we have observed no difference in the calorimetric behaviour of the two molecules. However, when a substituent on the alcohol function of the aromatic ring in the benzomorphane structure is present on the codeine and ethylmorphine molecules, a correlation between the shift of T_m towards lower temperatures and the increasing length of the substituent at this position can be observed. Thus, when there is no substituent (morphine and naloxone), no significant effect on the T_m was detected. In contrast, the presence of a methoxy group (codeine) induces a noticeable effect which is more remarkable when an ethoxy group (ethylmorphine) is in place. Consequently, the interaction of codeine and ethylmorphine with the model phospholipid membrane studied seems very dependent on the presence of a methyl or ethyl group on the hydroxyl function of the benzomorphane structure. Moreover, the lack of interaction observed with morphine and naloxone seems to correlate with the lack of a hydroxyl substituent on this position.

ACKNOWLEDGEMENTS

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